STEROIDS. XIV*

Preparation of Steroid Sulphate Salts

By

M. MARIÁN, B. MATKOVICS and SZ. J. VARGHA

Institute of Animal Physiology, Biochemical Research Group,
Attila József University, Szeged

(Received December 31, 1970)

Pyridinium and sodium salts of dehydroepiandrosterone, testosterone, oestrone, 17α-hydroxy-11β-deoxycorticosterone-21- and cortisol-21-sulphates have been prepared. In all cases sodium salts have been isolated after the decomposition of pyridinium salts.

The steroid sulphates play an important role in the living organism, in the metabolism of steroid compounds. Starting from this fact, a selective method has been developed for the preparation of sulphate esters in the mentioned groups which contained one or sometimes more hydroxyl-groups on different carbon atoms. Steroid sulphate ester salts differ from their starting compounds in their water-solubility.

A considerable part of steroid alcohols in animal tissues metabolize in their sulphuric acid ester forms. Practically they are potassium, sodium, etc. salts of sulphuric acid esters.

In the biosynthesis of sulphuric acid esters *in vivo* the adenosine-3′,5′-diphosphate-5′-sulphonic acid anhydride ("active sulphate" [17]) and — according to more recent conceptions — the ascorbic acid-3-sulphate [2] in the adrenal gland take part.

Several suitable chemical methods for the synthesis of sulphates are known. If a steroid sulphonic acid salt or sulphates are to be prepared, then according to one of the following methods: *a)* the sterols are brought into reaction in pyridine with pyridine sulphate in the presence of acetic acid anhydride [3], or *b)* dicyclohexyl-carbodiimide and sulphuric acid (dissolved in dimethylformamide) are added to the dimethylformamide solution of the steroids [4]. *c)* A further possibility of the preparation of a sterol sulphonic acid ester consists in the reaction of a sterol with a triethyl-amine solution of sulphur trioxide. In this case a sterol sulphonic acid-triethyl-ammonium salt can be separated [5]. *d)* The most frequently used method is briefly the following: the sterols are mixed with chlorosulphonic acid or pyridine sulphur trioxide in pyridine solution and the formed salt (pyridinium sulphate salt of the sterol) is isolated [6]. The reaction takes place at room temperature or in

* Part XIII.: I. WEISZ-VINCZE, GY. SCHNEIDER, M. HALMOS, J. A. SZABÓ and K. KOVÁCS: Acta Phys. et Chem. Szeged **17**, 67 (1971).

benzene solution at 50—60 °C [7—10]. The isolated pyridinium salt can be converted into other sulphate salts or sulphates [4, 6, 8].

If a sterol has more then one hydroxyl group, these react at different rates, thus, the mentioned methods can be used for selective sulphuration, varying the reaction time and the circumstances.

In the course of our experiments, different methods were examined and compared concerning their applicability for our mentioned purposes. The most suitable experimental methods are described below.

Experimental

Melting points were measured by a Boetius "Mikroheiztisch". The given values are uncorrected (see Experimental part of [11, 12]).

$[\alpha]_D$ values were determined at 20 °C, in the given solvent and concentration.

IR spectra were measured with a Unicam SP 200 Spectrophotometer.

For thin layer chromatography (TLC), in general, silicagel G (Reanal) adsorbent was applied in a layer thickness of 0.25 mm, the solvent system applied was the organic phase of a 9:1 mixture of *n*-butanol and water. The spots on the dried TLC plates were developed by 25% sulphuric acid.

The general principle of the method applied for the synthesis of steroid-sulphates was the following: Sulphur trioxide-pyridinium salt was prepared by a reaction of pyridine and chlorosulphonic acid and a pyridine solution of sterol was added. The steroid-sulphate-pyridinium salts obtained were isolated and transformed by sodium sulphate solution to their sodium salts. The salt, isolated by petroleum ether precipitation was recrystallized. The method described above can be generally used in practice for preparing different sterols, in some cases, of course, with slight modifications.

Dehydroepiandrosterone-3β-sulphonic acid pyridinium salt (I)

0.3 ml chlorosulphonic acid was added dropwise to 5 ml pyridine by cooling the solution with ice and 1 g dehydroepiandrosterone dissolved in 20 ml pyridine was slowly added to the mixture. Then the mixture was allowed to stand at room temperature for about 45 minutes. The salt was precipitated by the addition of petroleum ether (if the substance did not precipitate sufficiently, it was stored in a refrigerator for a night). The precipitate was filtered and washed with petroleum ether, then dissolved in 20 ml chloroform to remove the pyridine-sulphuric trioxide contaminations which are not soluble in chloroform. From the chloroform solution the salt was precipitated again by addition of petroleum ether. The yield was good (95%). If necessary, the substance can be further purified by dissolving in chloroform and precipitating with petroleum ether. M.p.: 130—132 °C; $[\alpha]_D = +140° \pm 2°$ ($c = 1.0$; methanol); M: 447.51. Anal.: Calc.: $C_{24}H_{33}O_5NS$ C 63.34 H 7.42 N 3.12 S 7.15; Found: C 63.39 H 7.46 N 3.25 S 7.20. ν_{max}^{KBr} 3400, 3100 (OH; C:C), 1725 (C:O), 1640 (C:C; C:N), 1200, 1060 (O.SO_2.O) cm^{-1}.

Dehydroepiandrosterone-3β-sulphonic acid sodium salt (II)

1 g of I was suspended in 10 ml saturated Na_2SO_4 solution and the pH of the solution was slowly raised to 7.5 by addition of $NaHCO_3$ while the substance slowly dissolved. The solution was allowed to stand about 2 hours at room temperature. Meanwhile the sodium salt precipitated. The precipitate was filtered and washed with a small amount of water and then with petroleum ether. The salt was recrystallized several times from petroleum ether. The yield was 95—98%. M.p.: 170—171 °C; $[\alpha]_D = +140° \pm 2°$ (c = 1.0; methanol); M: 390.14. (Lit. m.p.: 219—33 °C for the K-salt [14]). Anal.: Calc.: $C_{19}H_{27}O_5SNa$ C 58.50 H 6.97 S 8.23; Found: C 58.55 H 7.02 S 8.30. ν_{max}^{KBr} 3500 (OH), 1730 (C:O), 1630 (C:C), 1250, 1220, 1070 (O.SO_2.O) cm^{-1}.

Testosterone-17β-sulphonic acid pyridinium salt (III)

Prepared in the same way as I. M.p.: 138—40 °C; $[\alpha]_D = +200° \pm 2°$ (c = 1.0; methanol); M: 447.5. Anal.: Calc.: $C_{24}H_{33}O_5NS$ C 64.34 H 7.42 N 3.12 S 7.15; Found: C 64.30 H 7.47 N 3.30 S 7.25. ν_{max}^{KBr} 3500, 3350, 3200, 3050 (OH; C:N; C:C), 1650, 1630, 1600 (C:C.C:O), 1270, 1230, 1060 (O.SO_2.O) cm^{-1}.

Testosterone-17β-sulphonic acid sodium salt (IV)

1 g of III was dissolved in 10 ml distilled water, and after adding 1 g $NaHCO_3$ to the solution, allowed to stand at room temperature for two hours; then it was dried in a vacuum exsiccator, over P_2O_5, until the solvent disappeared. The dry residue was dissolved in 25 ml anhydrous methanol, filtered and dried and recrystallized from methanol-ether several times. The yield was about 80%, m.p.: 365—70 °C; $[\alpha]_D = +40° \pm 2°$ (c = 1.0; methanol); M: 390.4. Anal.: Calc.: $C_{19}H_{27}O_5SNa$ C 58.50 H 6.97 S 8.23; Found: C 58.45 H 7.01 S 8.30. ν_{max}^{KBr} 3600, 3500 (OH; C:C), 1650 (C:O), 1260, 1080, 1010 (O.SO_2.O) cm^{-1}.

Oestrone-3-sulphonic acid pyridinium salt (V)

The procedure is the same as described in the case of I. Thus sulphonic acid pyridinium salt could be isolated with a yield of about 80—85%. M.p.: 198 °C; $[\alpha]_D = -41° \pm 2°$ (c = 0.5; methanol); M: 429.46. Anal.: Calc.: $C_{23}H_{27}O_5NS$ C 64.38 H 6.34 N 3.26 S 7.47; Found: C 64.42 H 6.38 N 3.34 S 7.49. ν_{max}^{KBr} 3350, 3050 (OH; C:C; C:N), 1710, 1630, 1610 (C:O; C:C), 1290, 1250, 1060 (O.SO_2.O) cm^{-1}.

Oestrone-3-sulphonic acid sodium salt (VI)

Starting from 1 g of V the same method as described in detail for II was used. The substance can be separated with a yield of about 80%. After recrystallization from methanol-ether, the m.p. of VI was 230 °C. M: 372.36 (Lit. m.p.: 229—31 °C [3]). Anal.: Calc.: $C_{18}H_{21}O_5SNa$ C 58.11 H 5.68 S 8.62; Found: C 58.15 H 5.72 S 8.70. ν_{max}^{KBr} 3450 (OH), 1710, 1615 (C:O; C:C), 1200, 1110 (O.SO_2.O) cm^{-1}.

17α-Hydroxy-11-deoxy-corticosterone-21-sulphonic acid pyridinium salt (VII) (REICHSTEIN S *21-sulphonic acid pyridinium salt)*

Prepared in the way described for I; however in this case the purification in chloroform was uneffective because the by-products are also soluble. The yield is about 70%; m.p.: 191—96 °C; M: 505.55. Anal.: Calc.: $C_{26}H_{35}O_7NS$ C 61.71

H 6.97 N 2.76 S 6.34; Found: C 61.75 H 7.01 N 2.82 S 6.44. ν_{max}^{KBr} 3400, 3350, 3050 (OH; C:C; C:N), 1710, 1650, 1630, 1600 (C:C.C:O), 1280, 1200, 1030 (O.SO_2.O) cm^{-1}.

17α-Hydroxy-11-deoxy-corticosterone-21-sulphonic acid sodium salt (VIII) (REICHSTEIN *S 21-sulphonic acid sodium salt*

1 g of the pyridinium salt VII was dissolved in 20 ml saturated aqueous sodium sulphate solution and the pH was adjusted to 7.5 with crystalline sodium hydrocarbonate. The solution was allowed to stand at room temperature for two or three hours. The precipitate was centrifugated and dissolved in dry methanol. The solution was filtered and evaporated. The isolated substance was dissolved again in 30 ml methanol and precipitated with about 60 ml dry ether under cooling with ice. By repeating the fractionated precipitation, a substance of high purity could be obtained. Yield: 85—87%; m.p.: 188—190 °C; $[\alpha]_D = +223° \pm 2°$ (c = 1.0; methanol); M: 448.45. Anal.: Calc.: $C_{21}H_{29}O_7SNa$ C 56.29 H 6.52 S 7.15; Found: C 56.32 H 6.57 S 7.25 ν_{max}^{KBr} 3400 (OH), 1710, 1650 (C:C.C:O), 1270, 1240, 1040 (O.SO_2.O) cm^{-1}.

It may occur that during the purification the sodium salt does not precipitate and, instead, an organic layer separates. This layer dried in vacuum exsiccator, as described earlier, can be purified by dissolving in methanol and precipitating by ether.

Cortisol-21-sulphonic acid pyridinium salt (IX)

The preparation of IX was also the same as described for I. The yield was about 70%; m.p.: 132—34 °C; M: 522.55. Anal.: Calc.: $C_{26}H_{35}O_8NS$ C 59.70 H 6.74 N 2.68 S 6.12; Found: C 59.72 H 6.76 N 2.70 S 6.22. ν_{max}^{KBr} 3450, 3050(OH; C:C), 1700, 1640, 1610 (C:O; C:C.C:O), 1240, 1050, 1040 (O.SO_2.O) cm^{-1}.

Cortisol-21-sulphonic acid sodium salt (X)

1 g of IX was dissolved in 10 ml distilled water and 1 g sodium hydrocarbonate was added. The solution was evaporated in a vacuum exsiccator and the residue puridied, as described previously, by dissolution in methanol and precipitation with ether. After repeated recrystallization the yield was about 90% (m.p.: 180—85 °C; $[\alpha]_D = -200° \pm 2°$ (c = 1.0; methanol); M: 463.45. Anal.: Calc.; $C_{21}H_{29}O_8SNa$ C 54.31 H 6.90 S 6.89; Found: C 54.36 H 6.92 S 6.70. ν_{max}^{KBr} 3450(OH)), 1710, 1660 (C:C.C:O), 1270, 1240, 1040 (O.SO_2.O) cm^{-1}.

Discussion

The methods detailed above are partly based on modifications of known methods and partly are newly developed procedures for the preparation and isolation of different sterol-sulphonic acid sodium salts. In every case, the sterol-sulphonic acid sodium salts were isolated, the sulphates being rather labile and decomposing easily. Most of the sterol-sulphonic acid salts described in the Experimental part have not been described as yet, or are known in the form of other salts. All sterol-sulphonic acid salts are very easily soluble in water, therefore they are very suitable materials for different incubation purposes (incubation with different tissue homogenizates). It is very easy to isolate and hydrolyse the sterols from the sulphonic acid sodium

salts after incubation. The method of hydrolysis was the following: 50 ml of the sodium salt was dissolved in 2 N sulphuric acid and extracted with about 60—100 ml ethyl acetate and 150—200 ml ether. (Before extraction the ether was saturated with water). The collected ethyl acetate and ether was dried by sodium sulphate, filtered and evaporated. The dry residue was suitable for identification.

It is known from literature [7] that in the case of REICHSTEIN S and cortisone only the 21-hydroxy group reacts with chlorosulphonic acid under the conditions described.

* * *

The authors express their gratitude to the staff of the Microanalytical Laboratory of the Institute of Organic Chemistry, Attila József University Szeged, for the analyses and to the Gedeon Richter Pharmaceutical Works (Budapest) for the starting material supplied.

References

[1] *Nose, Y., F. Lipmann:* J. Biol. Chem. **233,** 1348 (1958).
[2] *Chu, T. M., W. R. Jr. Slounwhite:* Steroids **12,** 311 (1968).
[3] *Levitz, M.:* Steroids **1,** 177 (1963).
[4] *Mumma, R. O., C. P. Hoiberg, W. W. Weber II,:* Steroids **14,** 67 (1969).
[5] *Dussa, J. P., J. P. Joseph, S. Bernstein:* Steroids **12,** 49 (1968).
[6] *Sobel, A. E., P. E. Spoerri:* J. Amer. Chem. Soc. **63,** 1259 (1941).
[7] *Kornel, L., J. W. Kleber, J. W. Conine:* Steroids **4,** 67 (1964).
[8] *Baulieu, E. E., R. Emiliozzi:* Bull. Soc. Chim. Biol. **44,** 823 (1962).
[9] *Butenandt, A., H. Hofstetter:* Z. Physiol. Chim. **259,** 222 (1939).
[10] *Kirdani, R. Y.:* Steroids **6,** 845 (1965).
[11] *Matkovics, B., Gy. Göndös, Zs. Tegyey:* Magy. Kém. Foly. **72,** 303 (1966).
[12] *Matkovics, B., Zs. Tegyey, M. Resch, F. Sirokmán, E. Boga:* Acta Chim. Hung. **66,** 333 (1970).
[13] *Marián, M.:* Anal. Biochem. (in press).
[14] *Burstein, S., S. Lieberman:* J. Biol. Chem. **233,** 331 (1958).

СТЕРОИДЫ XIV

Получение Солей Сульфатов Стероидов

М. Мариан, Б. Матькович, С. Й. Варга

Пиридиновые и натриевые соли дегидроэпиандростерона тестостерона, эстрона, 17α-гидрокси-11β-дезоксикортикостерона-21 и кортизол-21-сульфата были приготовлены. Во всех случаях натриевые соли приготовились путём разложения пиридиновых солей.